DP Barcode: D353731 MRID No.: 47441204

DATA EVALUATION RECORD
ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE SHRIMP
850.1035

1. **CHEMICAL:** Ethylenethiourea (degradate of Metiram/Mancozeb) PC Code No.: 600016

2. **TEST MATERIAL:** Ethylenethiourea Purity: 100%

3. **CITATION:** Soucy, K. (2008) Ethylenethiourea-Acute Toxicity to Mysids (Americamysis bahia) Under Static conditions, Following OPPTS Guideline 850.1035. Project Number: 13921/6103, 2007/03. Unpublished study prepared by Springborn Smithers Laboratories. **Sponsor**: EBDC/ETU Task Force c/o McDermott, Will and Emery LLP 600 13th Street NW Washington, DC 20005
DP Barcode: D353731

4. **REVIEWED BY:** John Marton, Staff Scientist, Cambridge Environmental, Inc.

Signature: **Date:** 07/23/08

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental, Inc.

Signature: **Date:** 03/03/09

5. **APPROVED BY:** Brian Montague, Fishery Biologist USEPA

Signature: **Date:** February 23, 2015

Secondary Review by: Allen W. Vaughn **Date:** 04/10/15

6. **DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to shrimp. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. STUDY PARAMETERS

Age or Size of Test Organism:	<24 Hrs
Definitive Test Duration:	96 Hours
Study Method:	Static
Type of Concentrations:	Mean measured

8. CONCLUSIONS:

Results Synopsis

LC_{50}: 7.8 mg ai/L 95% C.I.: 5.1-10.9 mg ai/L

NOAEC: 3.0 mg ai/L

Probit Slope: N.A. Moving Average estimate used

9. ADEQUACY OF THE STUDY

A. Classification: Acceptable

B. Rationale: No major deviations were noted that would affect the reported results

C. Repairability: N.A.

10. BACKGROUND

11. GUIDELINE DEVIATIONS: This study was conducted following guidelines outlined in U.S. EPA OPPTS 850.1035 "Mysid Acute Toxicity Test." The following deviations from OPPTS 850.1035 were noted:

1. Pre-test health and mortality of the brood culture were not reported.
2. DO saturation dropped below 60% in replicate B of the nominal 25 mg ai/L treatment level at 48 hours; therefore, gentle, oil-free aeration was initiated.

These deviations do not impact the integrity or acceptability of the study.

12. SUBMISSION PURPOSE: This study was submitted to provide data on the effects to *Americamysis bahia* following acute exposure to ethylenethiourea for the purpose of chemical re-registration.

13. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are *Americamysis bahia*, *Penaeus setiferus*, *P. duorarun*, *P. aztecus* and *Palaemonetes sp.*	*Americamysis bahia*
Age Juvenile, mysids should be < 24 hours old	<24 hours
Supplier	In-house cultures. Brood stock originally from Aquatic Bio Systems, Inc. CO.
All shrimp are from same source?	Yes
All shrimp are from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period minimum 10 days	Brood culture was continuously maintained under conditions similar to test conditions.
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	None Reported
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study and no feeding for 24 hour before the beginning of the test if organisms are over 0.5 g each. Mysids should be fed throughout the study.	Mysids were fed live brine shrimp (*Artemia salina*) nauplii, *ad libitum*, twice daily.

Guideline Criteria	Reported Information
Pretest Mortality <3% mortality 48 hours prior to testing	Not Reported

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water	The dilution water was prepared by filtering natural seawater collected from the Cape Cod Canal, Bourne, Massachusetts. The water was diluted to a salinity of 20±3‰ with laboratory well water. The water was then pumped into holding tanks through a series of polypropylene core filters (20- and 5-micron).
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 (parts per thousand) for marine (stenohaline) shrimp and 10-17 for estuarine (euryhaline) shrimp, weekly range < 6	20‰
Water Temperature Approx. 22 ± 1 EC	24-25°C
pH 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	7.3-7.9
Dissolved Oxygen Static: ∃ 60% during 1st 48 hrs and ∃ 40% during 2nd 48 hrs, Flow-through: ∃ 60%	4.0-8.4 mg/L DO saturation dropped below 60% in replicate B of the nominal 25 mg ai/L treatment level at 48 hours; therefore, gentle, oil-free aeration was initiated.

Guideline Criteria	Reported Information
Total Organic Carbon Should be <5 mg/L in reconstituted seawater	0.36 mg/L (March 2008)
Test Aquaria 1. Material: Glass or stainless steel 2. Size: 19.6 L is acceptable for organisms ∃ 0.5 g (e.g. pink shrimp, white shrimp, and brown shrimp), 3.9 L is acceptable for smaller organisms (e.g. mysids and grass shrimp). 3. Fill volume: 15 L is acceptable for organisms ∃ 0.5 g, 2-3 L is acceptable for smaller organisms.	1. Glass beakers 2. 1 L 3. Test vessels were filled with 0.9 L of test solution.
Type of Dilution System Must provide reproducible supply of toxicant	N/A; test was conducted under static conditions
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	N/A
Biomass Loading Rate Static: # 0.8 g/L at # 17EC, # 0.5 g/L at > 17EC; flow-through: # 1 g/L/day (N/A for mysids)	N/A
Photoperiod 16 hours light, 8 hours dark	16 h light, 8 h dark; sudden transitions from light to dark, and vice-versa, were avoided. The test area was illuminated with fluorescent bulbs at an intensity of 47-77 footcandles (510-830 lux) at the solutions' surface.

Guideline Criteria	Reported Information
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Solvent: N/A Maximum conc.: N/A

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 shrimp, then no definitive test is required.	Preliminary range-finding tests were conducted with mysids <24 hours and 5-6 days old. See Reviewer's Comments section for further details.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.	1.6, 3.1, 6.3, 13, 25 and 50 mg ai/L
Number of Test Organisms Minimum 20/level, may be divided among containers	20 per control and level, equally divided among 2 replicates
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1EC 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	1. Temperature was measured daily in each test vessel. Additionally, temperature was continuously monitored in replicate B of the nominal 25 mg ai/L treatment level. 2. DO and pH were measured daily in each test vessel.

Guideline Criteria	Reported Information
Chemical Analysis needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Samples were collected at 0 and 96 hours and analyzed using HPLC with UV detection. The method validation established an average recovery of 93.4%±8.38% from 0.1% trifluoroacetic acid in 20 ppt filtered seawater. At test initiation, samples were removed from the intermediate vessel prior to division into the replicate test vessels, and at test termination samples were removed from a composite of replicates A and B.

14. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with all pertinent U.S. EPA Good Laboratory Practice regulations (40 CFR, Part 160) with the following exceptions: routine dilution water and food contaminant screening analyses for pesticides, PCBs and toxic metals were conducted at GeoLabs, Inc., Braintree, Massachusetts using standard U.S. EPA Procedures and are considered facility records. Since the analyses were conducted following standard validated methods, these exceptions had no impact on the study results.
Recovery of Chemical	93-110% of nominal Mean measured concentrations were 1.5, 3.0, 6.4, 14, 26 and 53 mg ai/L

Guideline Criteria	Reported Information
<u>Control Mortality</u> Not more than 10% of control organisms may die or show abnormal behavior.	5% (one mysid died)
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (mg ai/L)		Number of Shrimp	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	<0.15	20	0	0	0	1
1.6	1.5	20	1	1	1	1
3.1	3.0	20	0	0	0	0
6.3	6.4	20	0	1	2	3[a]
13	14	20	9	14	15	18
25	26	20	8	20	20	20
50	53	20	8	20	20	20

[a] Lethargy reported in a number of surviving adults

Other Significant Results:

The study author reported that the 5% mortality at the lowest treatment level was within the expected range of naturally occurring variability; therefore, it is not considered an adverse response from exposure to the test substance.

All surviving mysids at the control and mean-measured 3.0 mg ai/L treatment level appeared normal and healthy throughout the 96-hour exposure period. At 72 and 96 hours, several surviving mysids at the mean-measured 6.4 mg ai/L treatment level appeared lethargic. At 72 and 96 hours, all surviving mysids at the mean-measured 14 mg ai/L treatment level were lethargic.

B. Statistical Results

Method: The LC_{50} and 95% C.I. for each observation interval were determined using the binomial probability. The statistical NOAEC value was determined using the Kruskal-Wallis Test. Data were first checked for normality using Chi-Square Test (Weber et al., 1989) and for homogeneity of variance using Bartlett's Test (Horning and Weber, 1985). These statistical determinations were performed using TOXSTAT® Version 3.5 (Gulley et al., 1996).

96-hr LC_{50}: 9.2 mg ai/L 95% C.I.: 6.4-14 mg ai/L
NOAEC: 3.0 mg ai/L
Probit Slope: Not Applicable

15. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC_{50} (C.I.)	9.2 (6.4-14) mg ai/L
Moving Average Angle LC_{50} (95% C.I.)	7.8 (5.1-10.9) mg ai/L
Probit LC_{50} (95% C.I.)	8.3 (0- Infinity) mg ai/L
Probit Slope	4.1 (-2.7-11.0)
NOAEC-observed	3.0 mg ai/L

16. REVIEWER'S COMMENTS:

The reviewer's statistical NOAEC value was identical to the study author's NOAEC value. However, lethargy was observed in the 6.4 mg ai/L concentration along with the small number of mortalities (15 %). Thus, the observed NOAEC is 3.0 mg ai/L. The reviewer's $LC_{50,}$ using the Moving Average Method, was lower and associated with a narrower 95% confidence interval than that reported by the study author. Therefore, the reviewer's results are reported in the Conclusions section of this DER.

A preliminary range-finding study was conducted under static conditions at nominal concentrations of 0.010, 0.10, 1.0, 10 and 100 mg ai/L, and a control using myids <24 hours old and 5-6 days old. One replicate per age group (10 mysids per replicate) was

established for each treatment level and the control. After 96 hours of exposure, complete mortality was observed among mysids <24 hours old exposed to the 10 and 100 mg ai/L treatment levels. No mortality or adverse effects were observed among mysids exposed to any of the remaining treatment levels tested (0.010, 0.10 and 1.0 mg ai/L) or the control in the <24 hour old age class. Following 96 hours of exposure, 90 and 100% mortality was observed among mysids 5 to 6 days old exposed to the 10 and 100 mg ai/L treatment levels, respectively. Mortality was 10% for 5-6 day old mysids in the 0.010 and 0.10 mg ai/L treatment levels during this period. No mortality or adverse effects were observed among mysids exposed to the remaining treatment level tested (1.0 mg ai/L) or the control in the 5 to 6 days old age class. Since no difference in sensitivity was observed between the two age groups, juvenile mysids (<24 hours old) were used during the definitive exposure. Based on these results and consultation with the Study Sponsor, nominal concentrations of 0.63, 1.3, 2.5, 5.0 and 10 mg ai/L were chosen for the definitive exposure.

The in-life portion of the definitive exposure period was conducted from March 13 to March 17, 2008. Though there was a drop in oxygen levels in one replicate of the 25 mg/L test level, this was not felt to have affected the results as both replicates had already experienced 100 % mortality by 48 hours. No other departures were observed for this water quality parameter.

17. REFERENCES:

ASTM. 2002. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Road, West Conshohocken, PA 19428.

Gulley, D.D., Boelter, A.M. and Bergman, H.L. 1996 TOXSTAT® Release 3.5. University of Wyoming, Laramie, Wyoming.

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Stephan, C.E. 1982. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal Communication to Dr. Lowell Bahner, Chairman ASTM Task Force Group (E-47) on Calculating LC50s.

U.S. EPA. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1996a. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Guideline, OPPTS 850.1035. Mysid Acute Toxicity Test. "Public Draft". EPA 712-C-96-136. April 1996. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1996b. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Guideline, OPPTS 850.1000. Special Consideration for Conducting Aquatic Laboratory Studies. "Public Draft". EPA 712-C-96-113. April 1996. U.S. Environmental Protection Agency, Washington, DC

Weber, C.I., W.H. Peltier, T.J. Norberg-King, W.B. Horning II, F.A. Kessler, J.R. Menkedick, T.W. Heiheisel, P.A. Lewis, D.J. Klemm, Q.H. Pickering, E.L. Robinson, J.M. Lazorchak, L.J. Wymer and R.W. Freyberg (eds.). 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 2nd ed. EPA/600/4/89/001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY. THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
53	20	20	100	9.536742E-05
26	20	20	100	9.536742E-05
14	20	18	90	2.012253E-02
6.4	20	3	15	.1288414
3	20	0	0	9.536742E-05
1.5	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT 6.4 AND 14 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 9.151942

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
5	.1412274	7.762586	5.106347-10.85085

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	2.727625	12.67107	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 4.133365
95 PERCENT CONFIDENCE LIMITS =-2.693102 AND 10.95983

LC50 = 8.33616
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 4.108756
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	1	
1	4.5	20	1	
2	3.0	20	0	
3	**6.4**	**20**	**3**	
4	14	20	18	*
5	26	20	20	*
6	53	20	20	*